Chemical Cues in the Ocean

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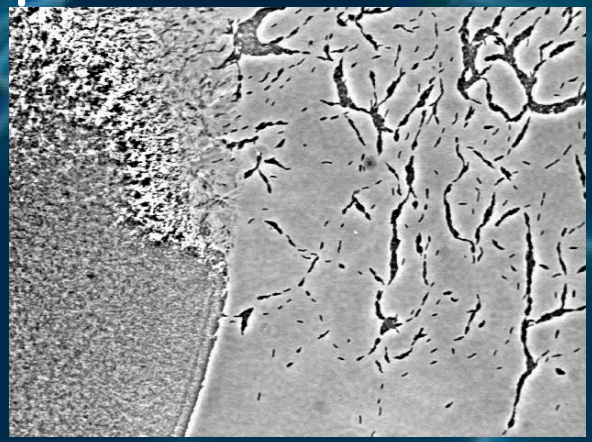
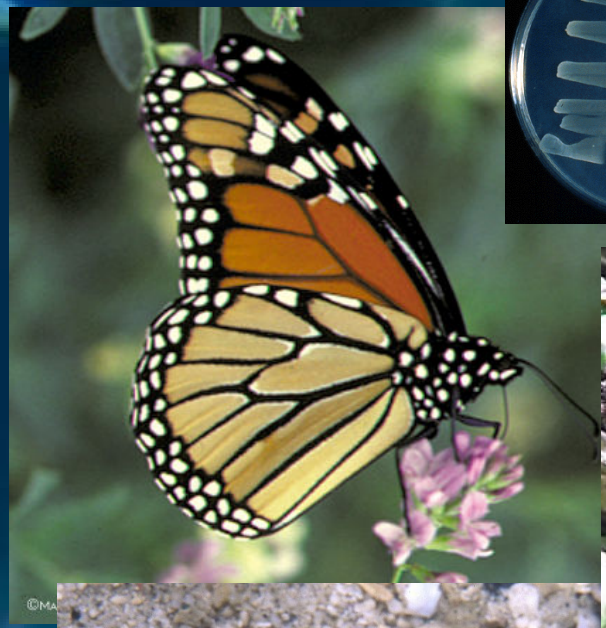
and School of Chemistry & Biochemistry

Organisms of all types are under natural selection pressure to avoid becoming food for others…



Some do this with chemical defenses..



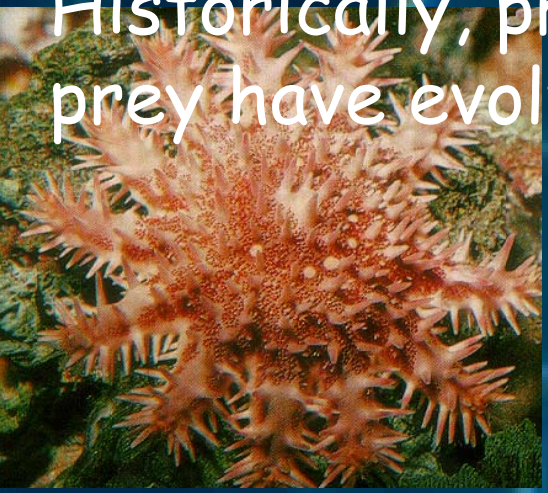
Corals, marine sponges, worms, seaweeds face heavy predation pressure

But herbivory and predation are actually healthy for coral reefs…

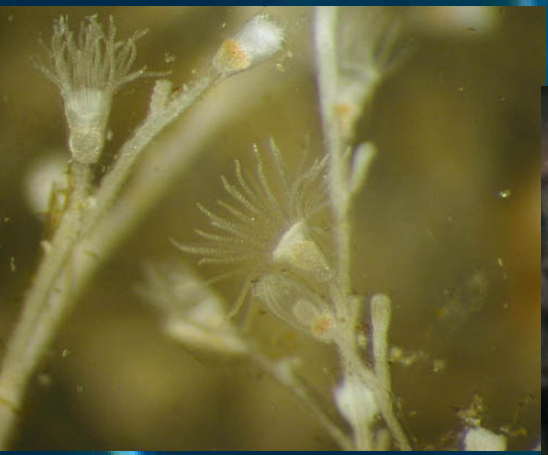
Coral reefs are under threat worldwide…

Probable factors include loss of herbivores, rising temperatures, storms, pollution, disease

Historically, predation has been intense – and so prey have evolved various anti-predatory defenses

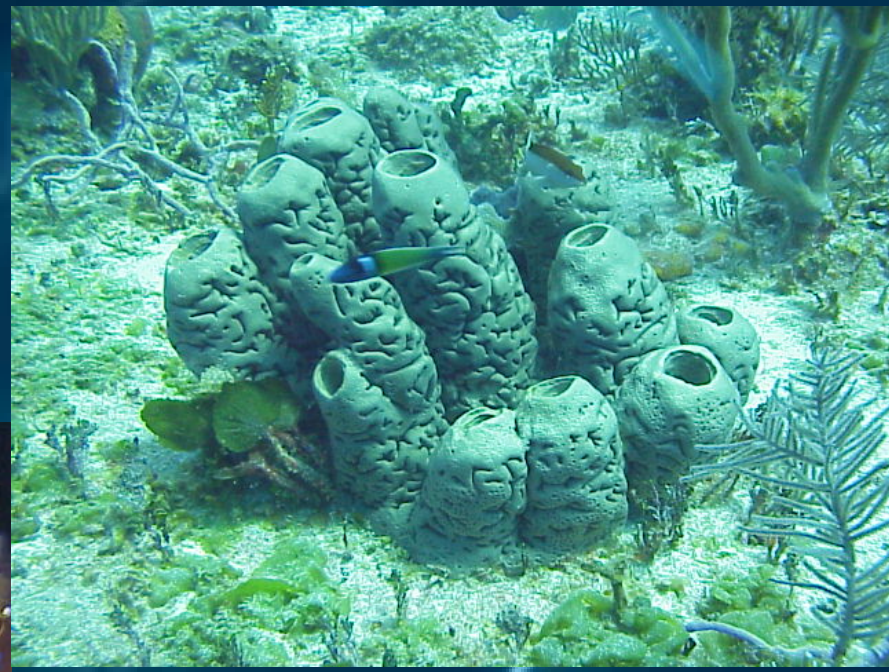




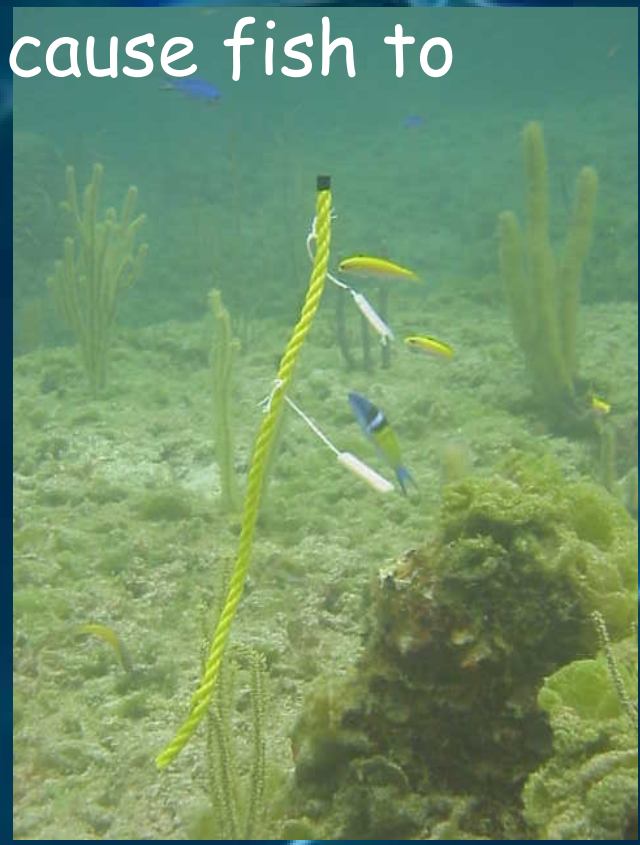





Marine sponges: great candidates for testing hypotheses re anti-predator chemical defenses…



Unpalatable compounds in sponges cause fish to reject food



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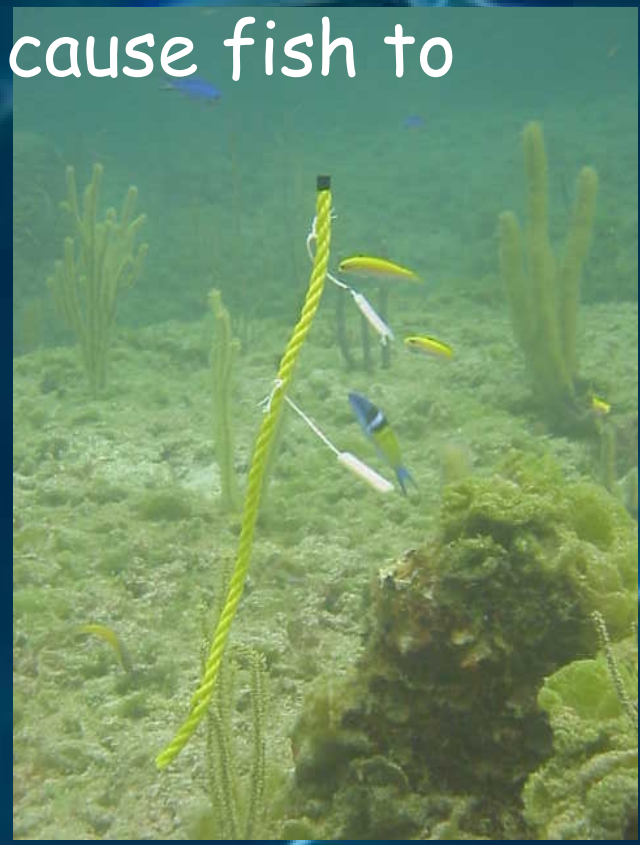
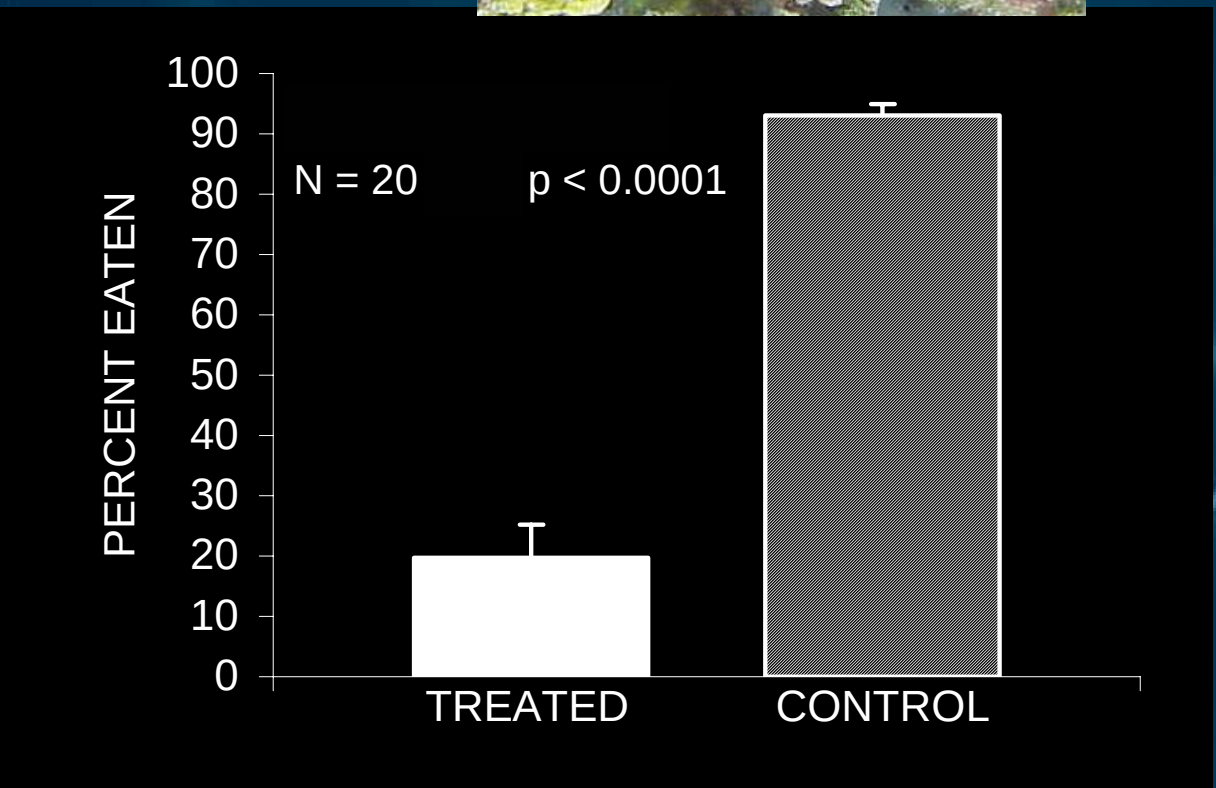
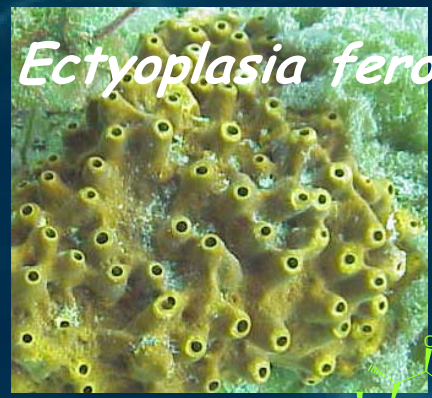
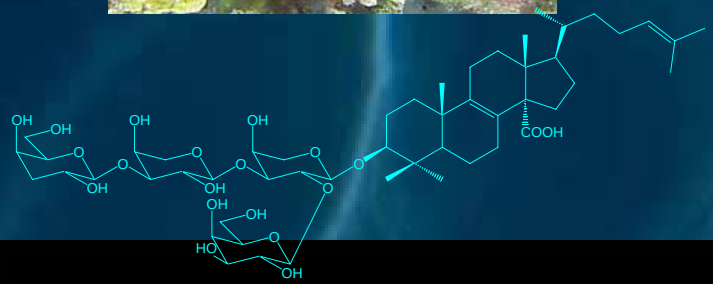
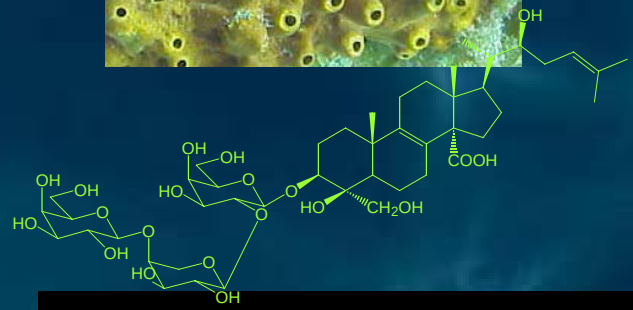


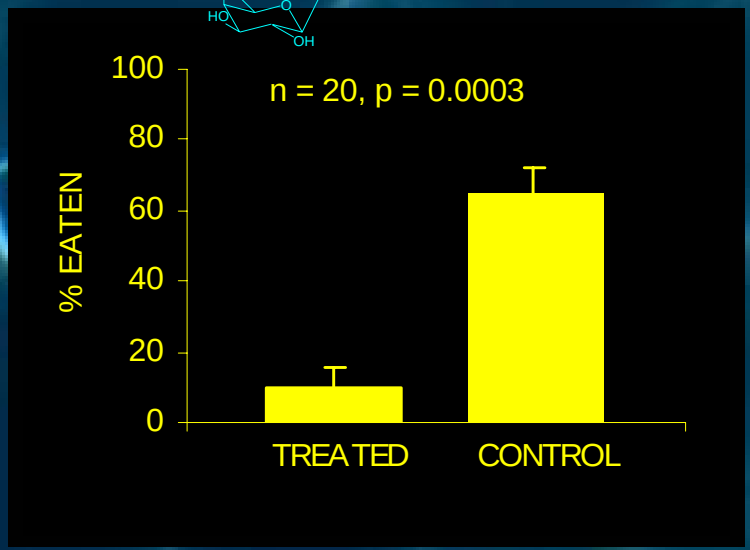


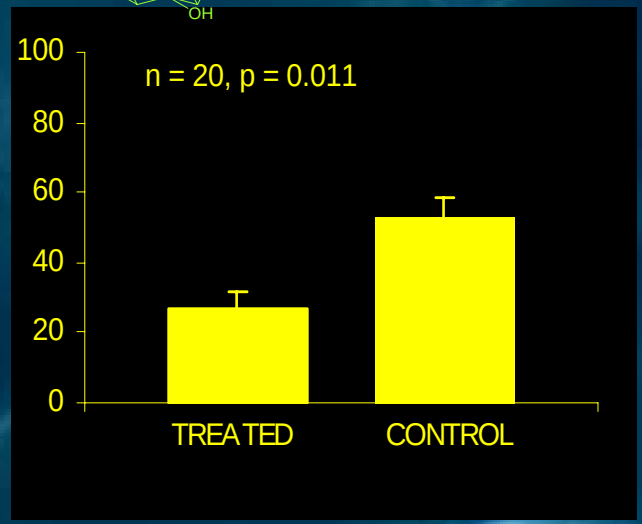
Predator deterrents in sponges







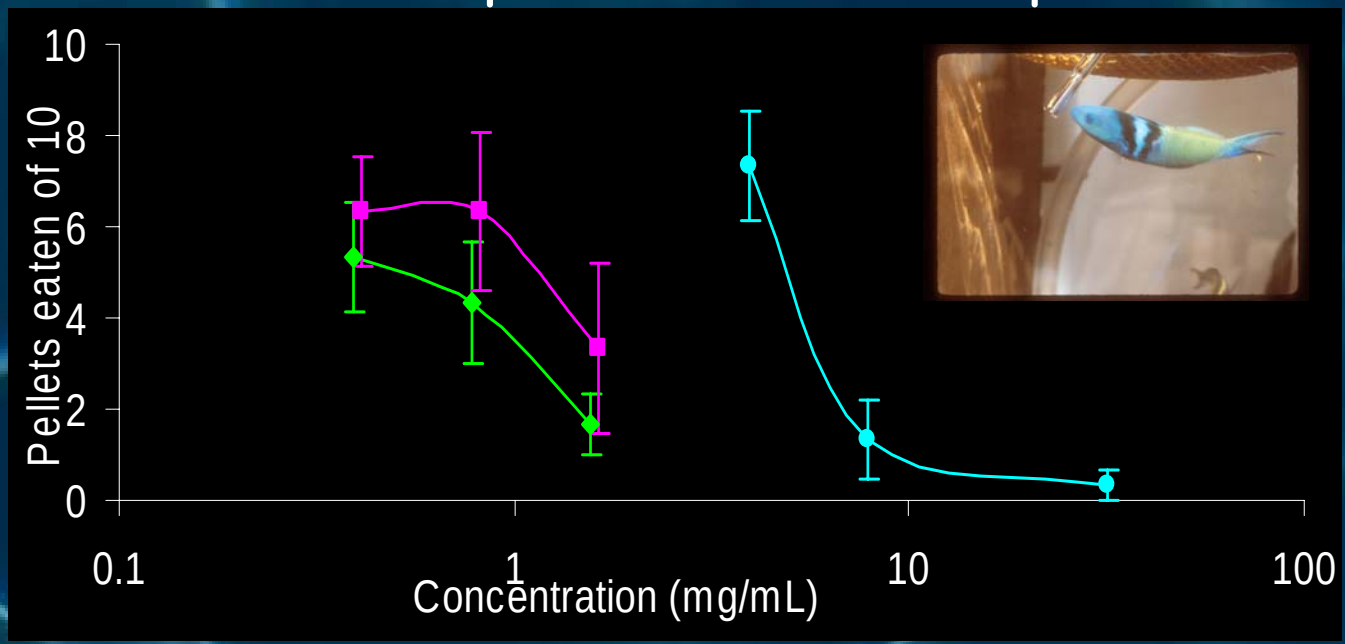


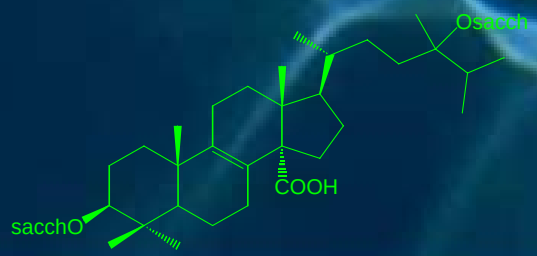


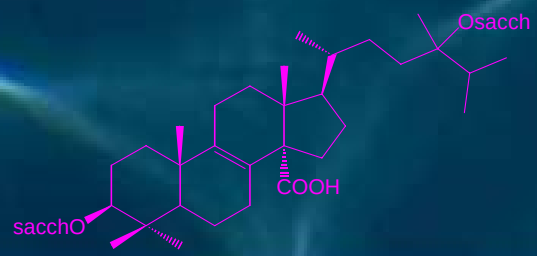
Kubanek, Pawlik, Eve, Fenical (2000) Mar Ecol Prog Ser 207:69-77
Kubanek, Pawlik, Fenical (2001) Nat Prod Lett 15:275-285

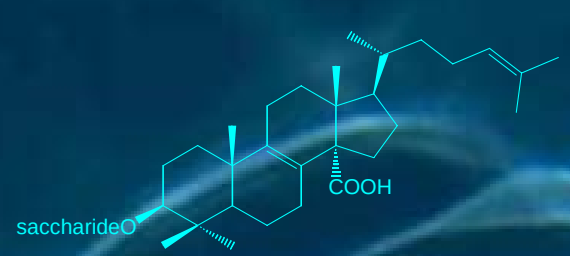
We know…

- that chemical defenses affect different predators differently
- concentration-dependence of individual compounds
- that rejection occurs within ~1 sec or less
  - involvement of specific chemoreceptors?





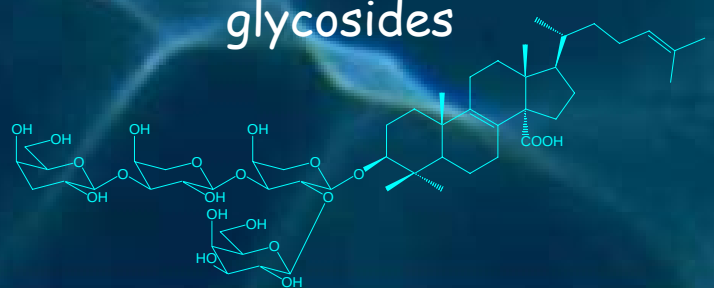




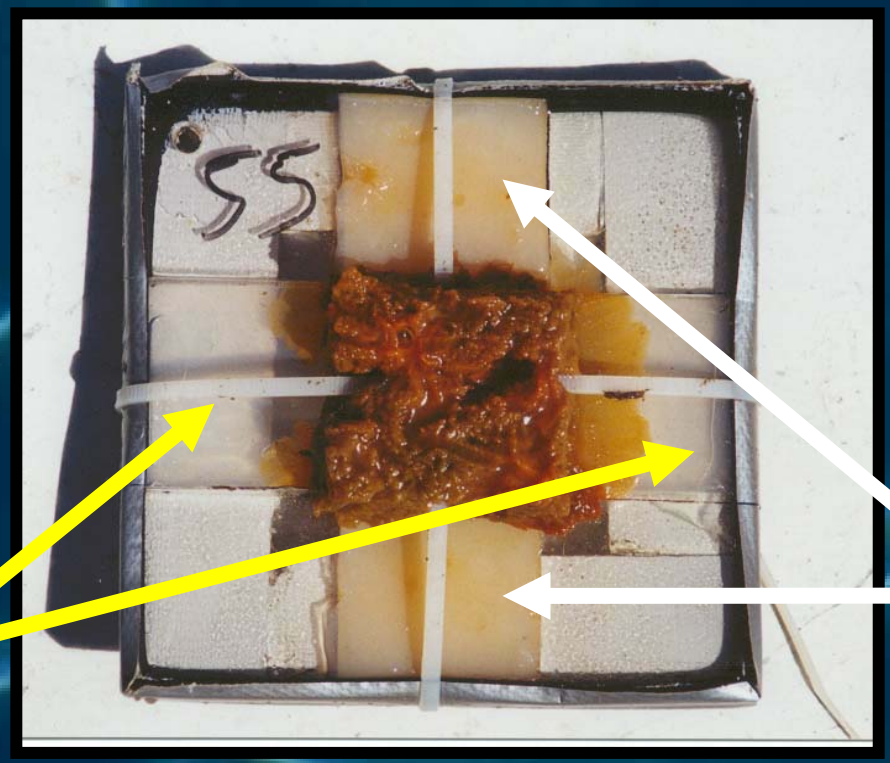
Some sponge compounds also deter settlement by algae and invertebrates

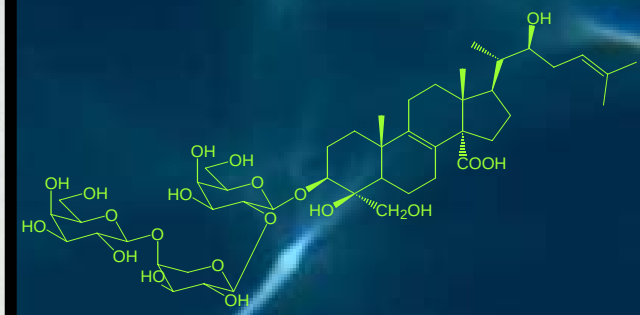
gel treated with sponge triterpene glycosides

control gel



Others sponge compounds deter overgrowth by neighboring sponges



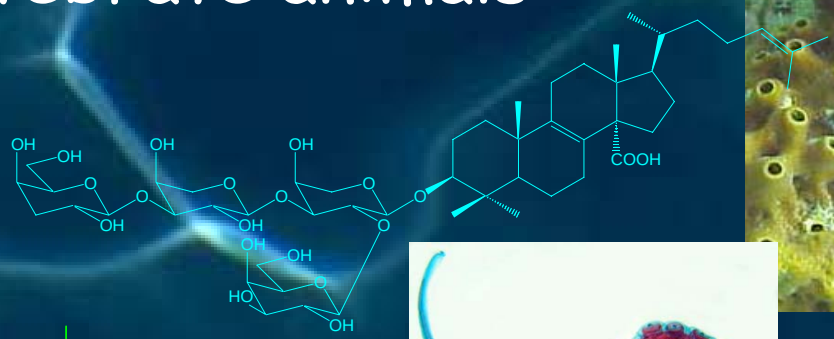


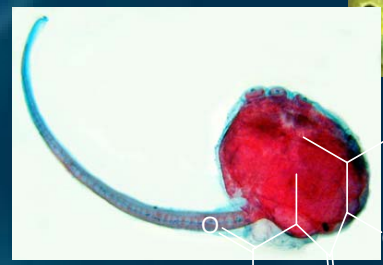
Control gel

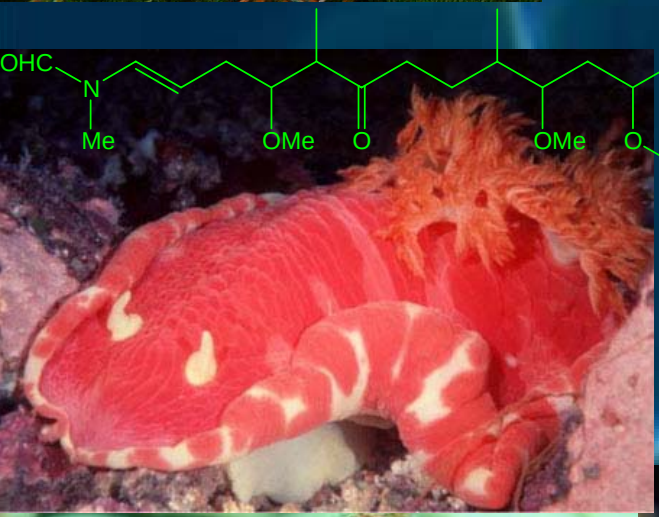
Gel treated with sponge triterpene glycosides

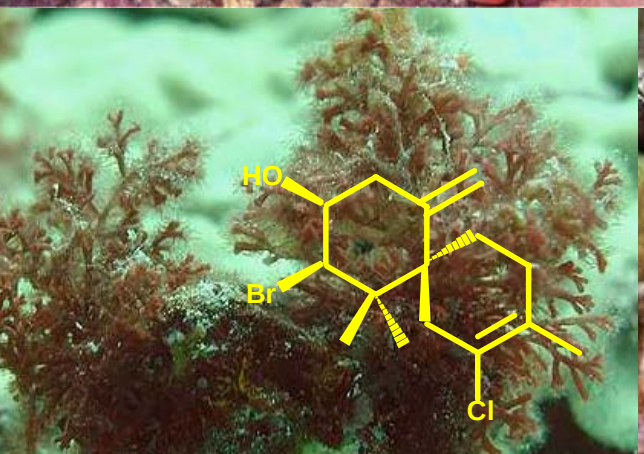
Kubanek, Whalen, Engel, Kelly, Henkel, Fenical, Pawlik (2002) *Oecologia* 131:125-136

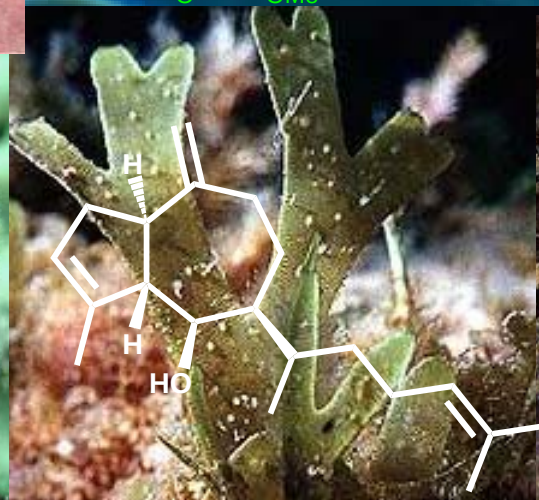
Chemical defenses are common among marine plants and invertebrate animals







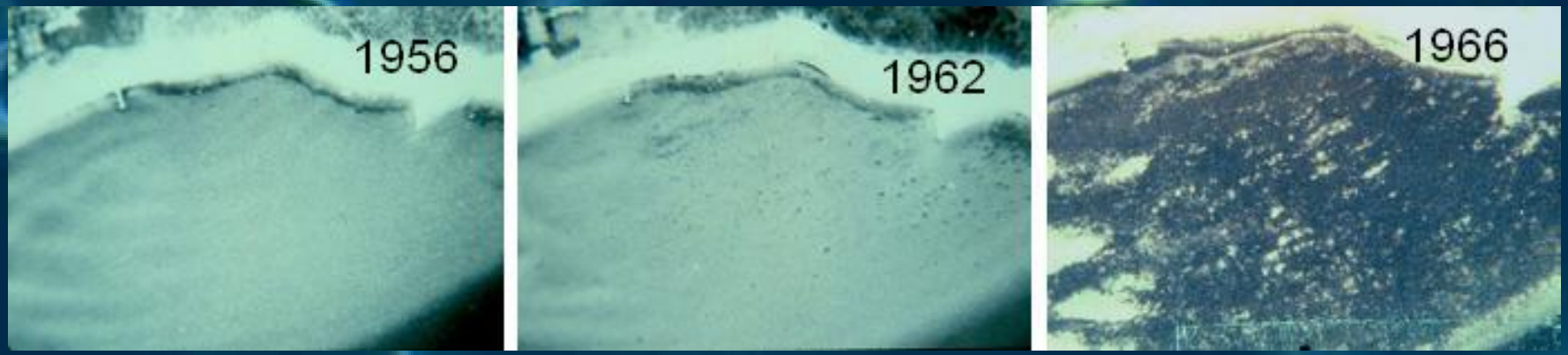




Pathogens are poorly understood but clearly affect coral reef communities

unknown disease decimated this herbivore in 1980's and led to widespread algal overgrowth of corals

Marine plants can also suffer from disease



1930's eelgrass wasting epidemic removed ~90% of North Atlantic eelgrass – recovery took decades

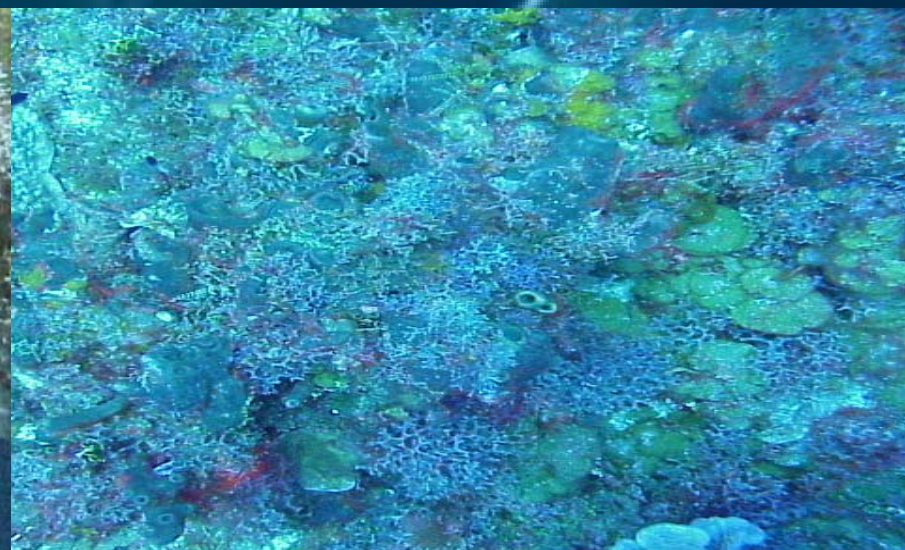
wasting disease on eelgrass

coralline lethal orange disease

Disease can cause losses of commercially important algal species

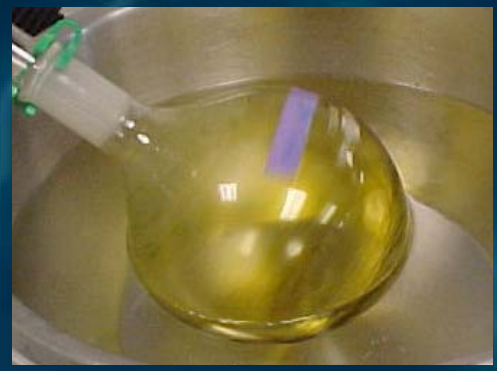
d

Seawater is full of potentially pathogenic microbes
– why aren't all seaweeds susceptible?
- natural antibiotics protect some seaweeds?



Testing seaweeds for antimicrobial chemical defenses

seaweed extraction



1) incorporation of seaweed extract into agar wells
2) inoculation with marine fungus

6 days for fungal growth

comparison with no-extract controls

ANTIMICROBIAL POTENTIAL OF 53 SEAWEEDS

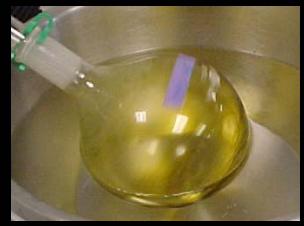
53 seaweed species collected in the Bahamas and tested against marine fungi and bacteria collected in same environment

- Over half of seaweed extracts deterred growth by at least 1 microbe
- Most showed specific activities
- YES, antimicrobial chemical defenses seem to be common and diverse


Lobophora variegata

BIOASSAY-GUIDED FRACTIONATION

Lobophora variegata extract



liquid-liquid partitioning

hexanes | chloroform | ethyl acetate | *n*-butanol | water-soluble

reversed phase silica

10-20% aqueous methanol eluted fraction

size exclusion chromatography

fractions 1-5
brown pigments

fractions 6-8
orange and yellow pigments
and galactolipids

fractions 9-30
green pigments

fractions 5-6

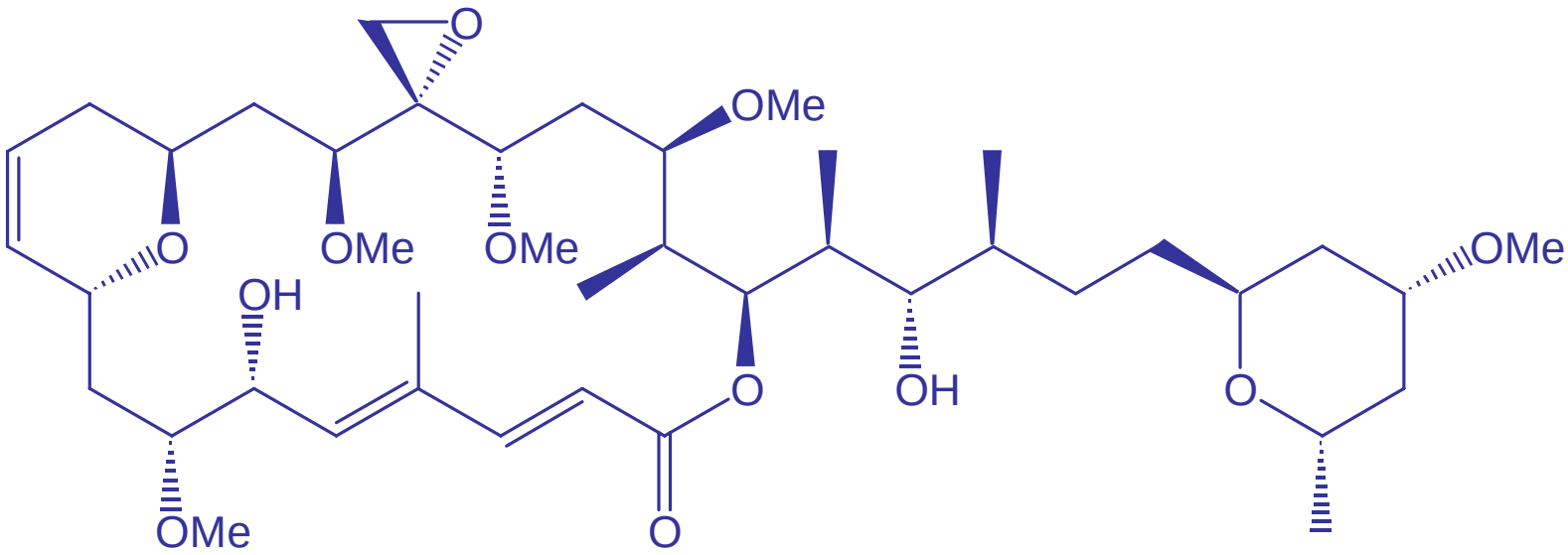
reversed phase HPLC

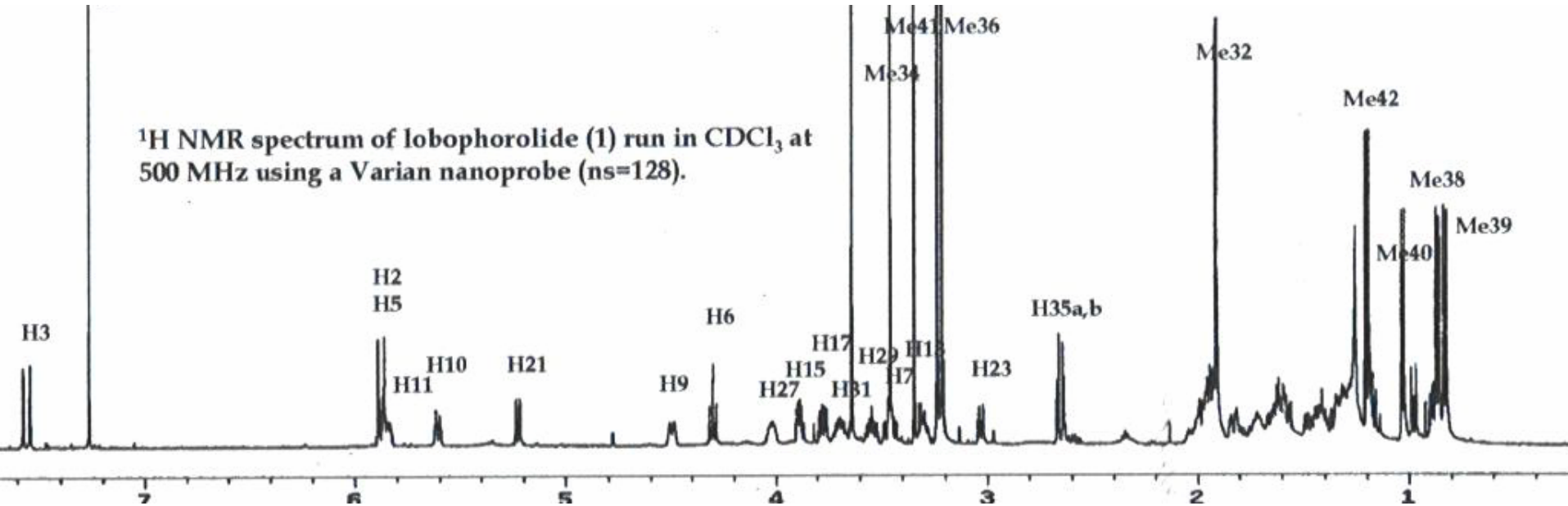
broad peak

repetitive normal phase HPLC

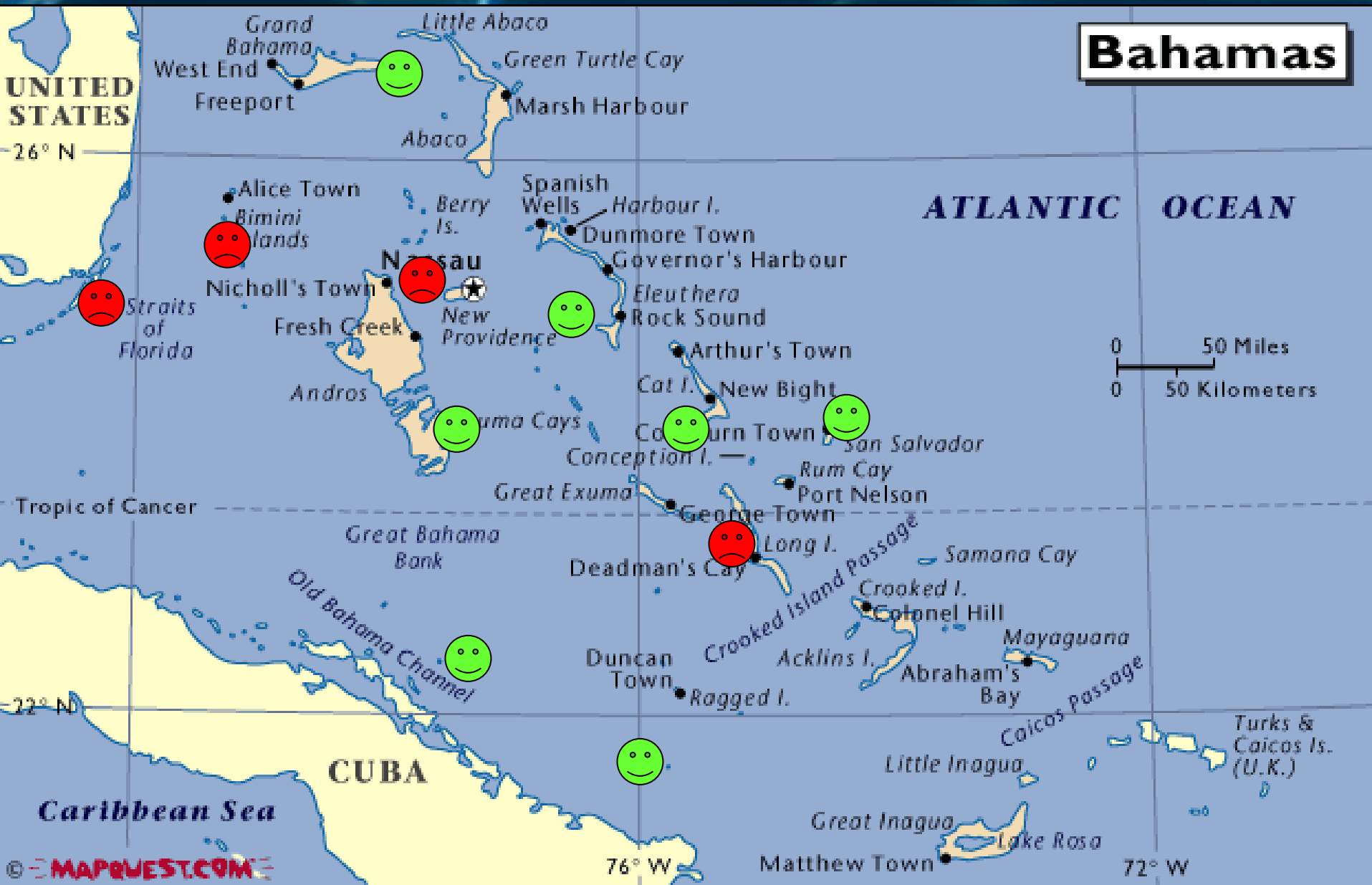
one antifungal compound
lobophorolide [approx. 200 μg (0.0002 % by dry weight)]

LOBOPHOROLIDE STRUCTURE





LOBOPHOROLIDE IN THE BAHAMAS AND FLORIDA (73 samples)

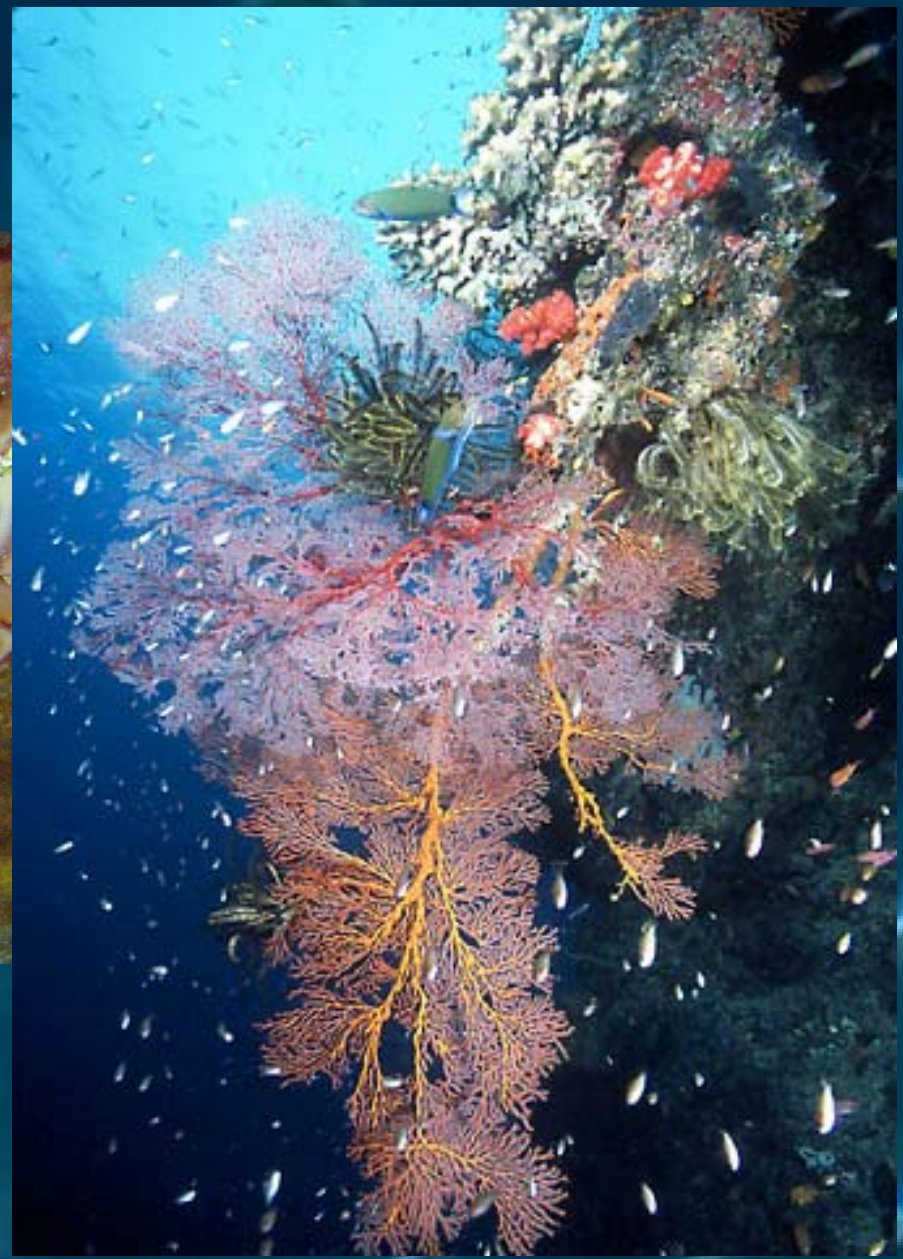


BIOMEDICAL POTENTIAL OF LOBOPHOROLIDE

Type of Bioactivity	Assay Organism	Bioactivity (μg/mL)
Antifungal	*Candida albicans*	MIC = 1.3
	C. albicans (amphotericin-resistant)	MIC = 0.5
Anticancer	human colon tumor (HCT-116)	IC_{50} = 0.03

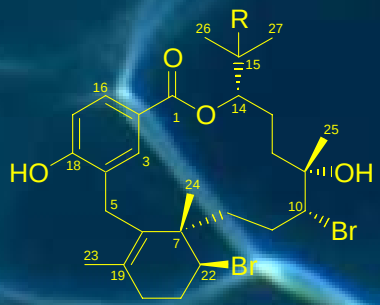
Kubanek, Jensen, Keifer, Sullards, Collins, Fenical (2003) PNAS 100:6916-6921

Drugs from the sea?

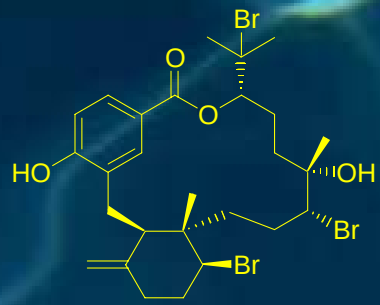


Drugs from the sea

Callophycus serratus from Fiji coral reefs



1 R=Br
2 R=OH



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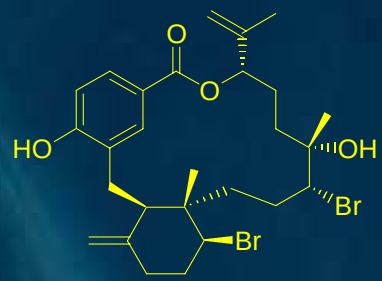

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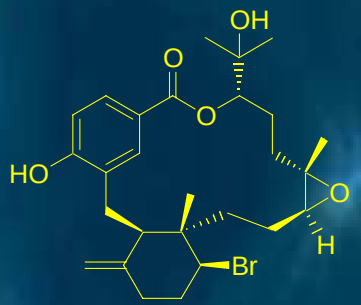

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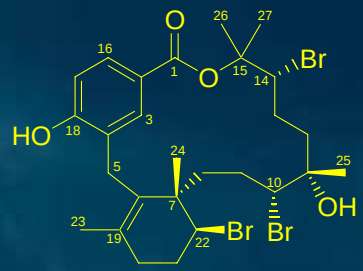

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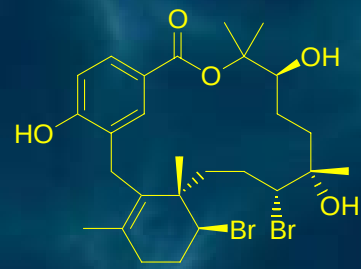

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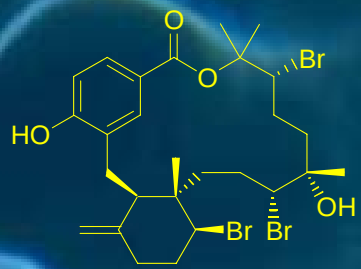

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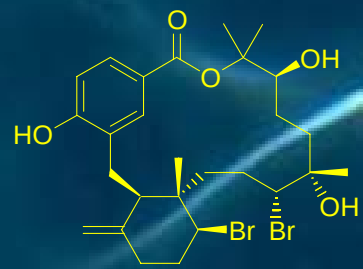

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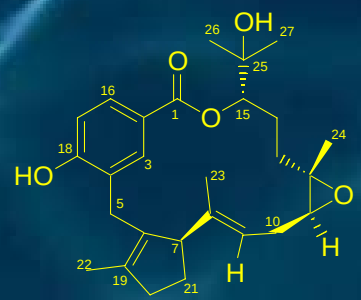


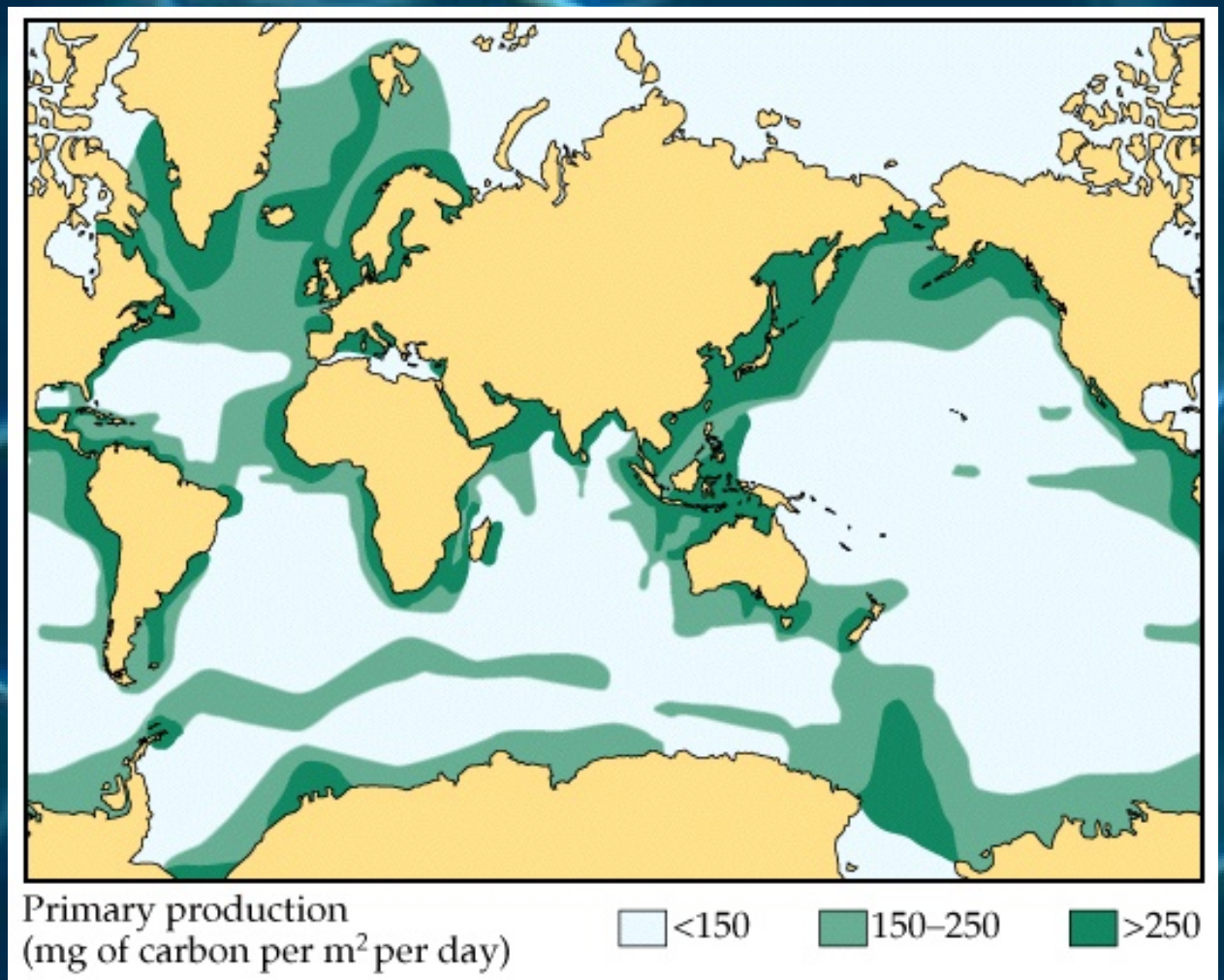
10

Kubanek, Prusak, Snell, Giese, Hardcastle, Fairchild, Aalbersberg, Raventos-Suarez, Hay (2005) Org. Lett. 7:5261-5264

Kubanek, Prusak, Snell, Giese, Fairchild, Aalbersberg, Hay (in press) J. Nat. Prod.

The Plankton…

- Greatest source of fixed carbon and the source of much of Earth's biologically available nitrogen



The Plankton…

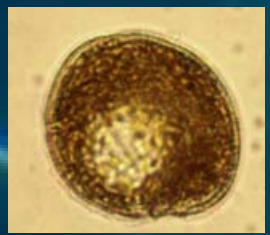
- Greatest source of fixed carbon and the source of much of Earth's biologically available nitrogen

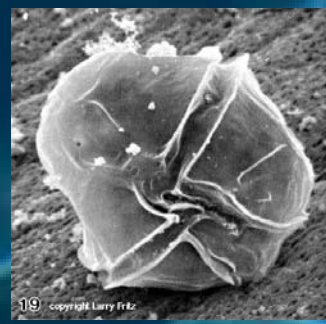
- Abundance of grazers (copepods, rotifers, etc.) plus microbial diversity: complex population and community interactions

- "Featureless" – large scale physical patterns of nutrients, temperature, light, fluid flow
  vs. small phytoplankton & zooplankton

- How important are chemical cues?

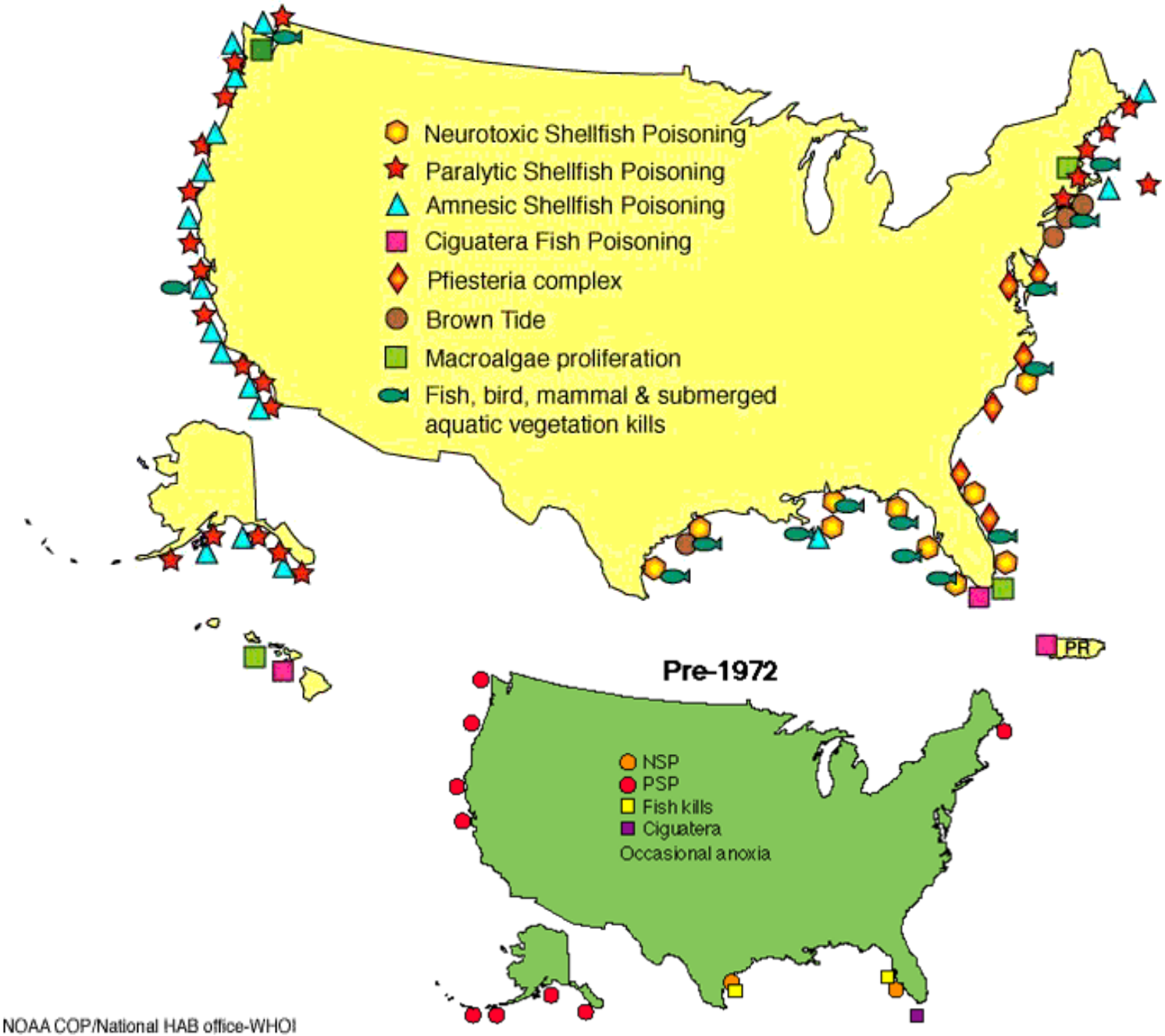
RED TIDES:
BLOOMS OF (TOXIC?) PHYTOPLANKTON





Direct costs to U.S. fisheries, tourism, monitoring, human health: $20M per bloom

Major HAB-related Events in the Coastal U.S.



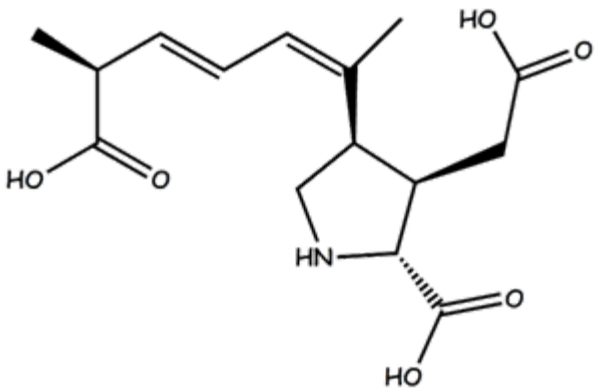
Source: NOAA COP/National HAB office-WHOI

Human health effects of red tides

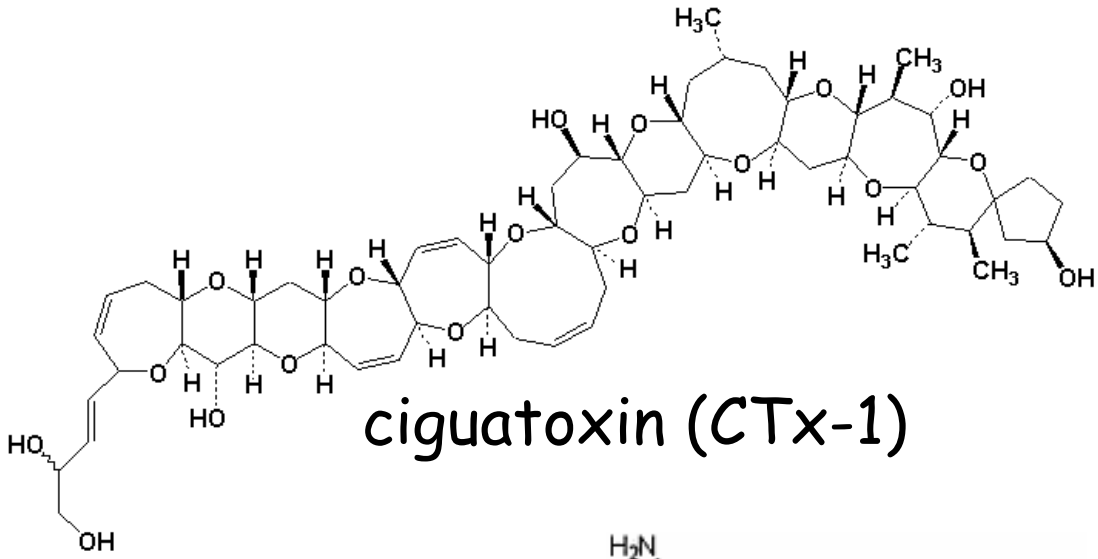
- Phytoplankton toxins cause gastrointestinal and neurological symptoms

- 3 modes of exposure
  - consumption of filter feeding bivalves
  - concentrated through food web to fish
  - inhalation of airborne toxins

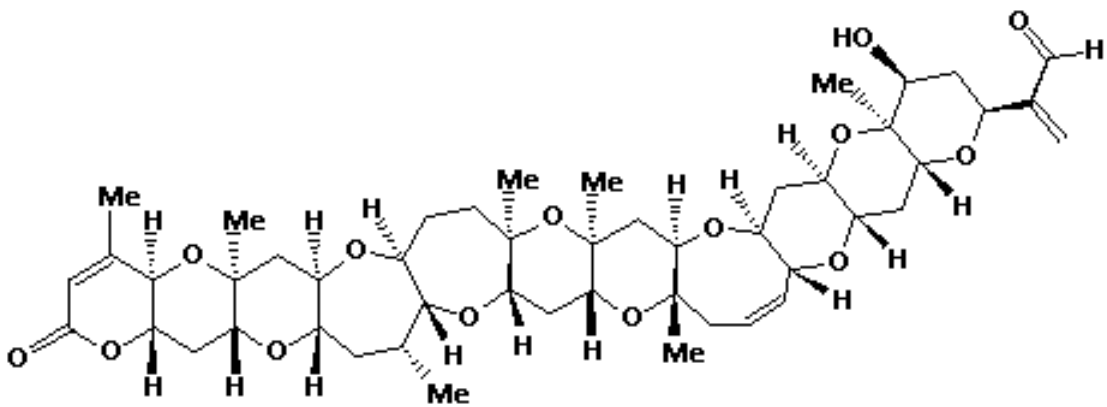
POTENT NEUROTOXINS PRODUCED BY PHYTOPLANKTON



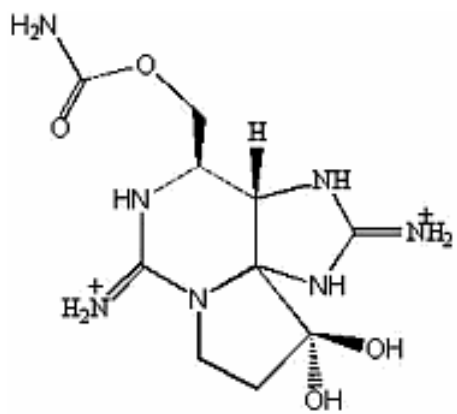
domoic acid



ciguatoxin (CTx-1)



brevetoxin B (PbTx-2)



saxitoxin



- Phytoplankton neurotoxins don't have the same effects on all organisms

- Not clear how neurotoxins affect predators and competitors

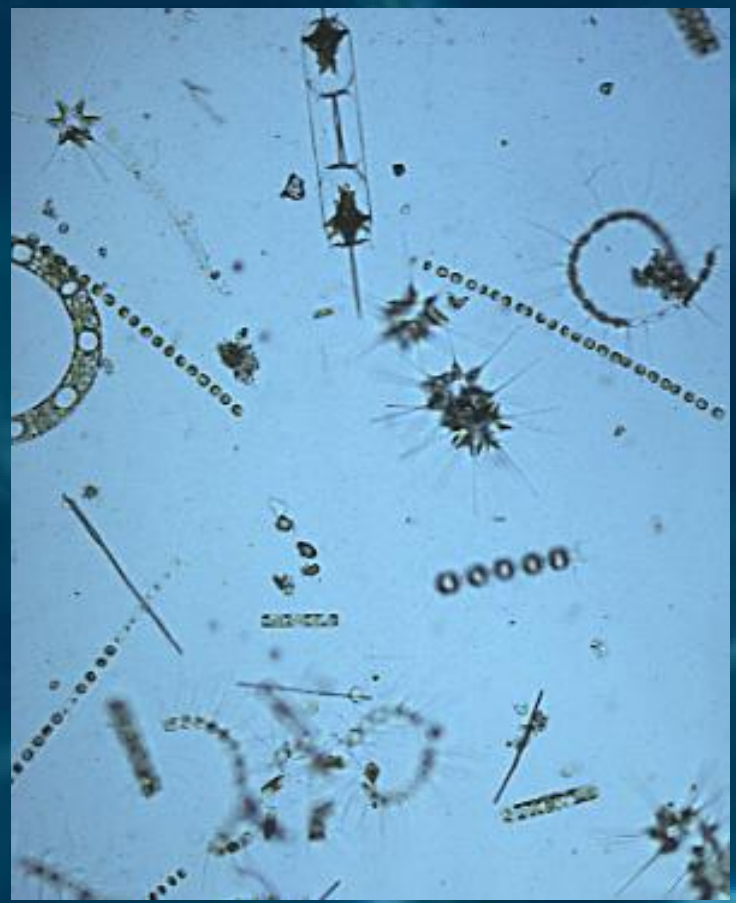
- How can some phytoplankton bloom

at a million cells per liter or more?

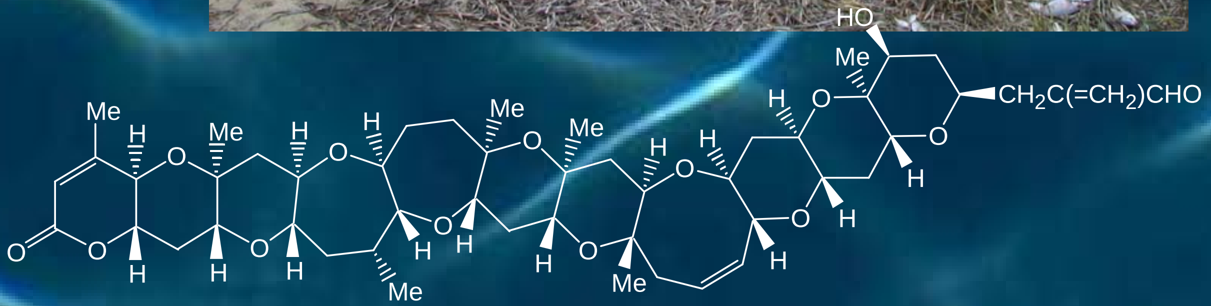
  - do they use toxins to escape predation?
  - to exclude competitors?

Hypothesis:

Red tide phytoplankton are engaging in chemical warfare against other phytoplankton



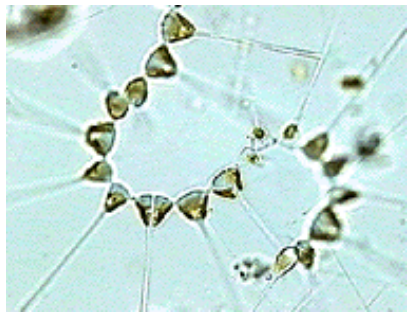
MODEL SYSTEM: FLORIDA RED TIDE DINOFLAGELLATE *Karenia brevis*



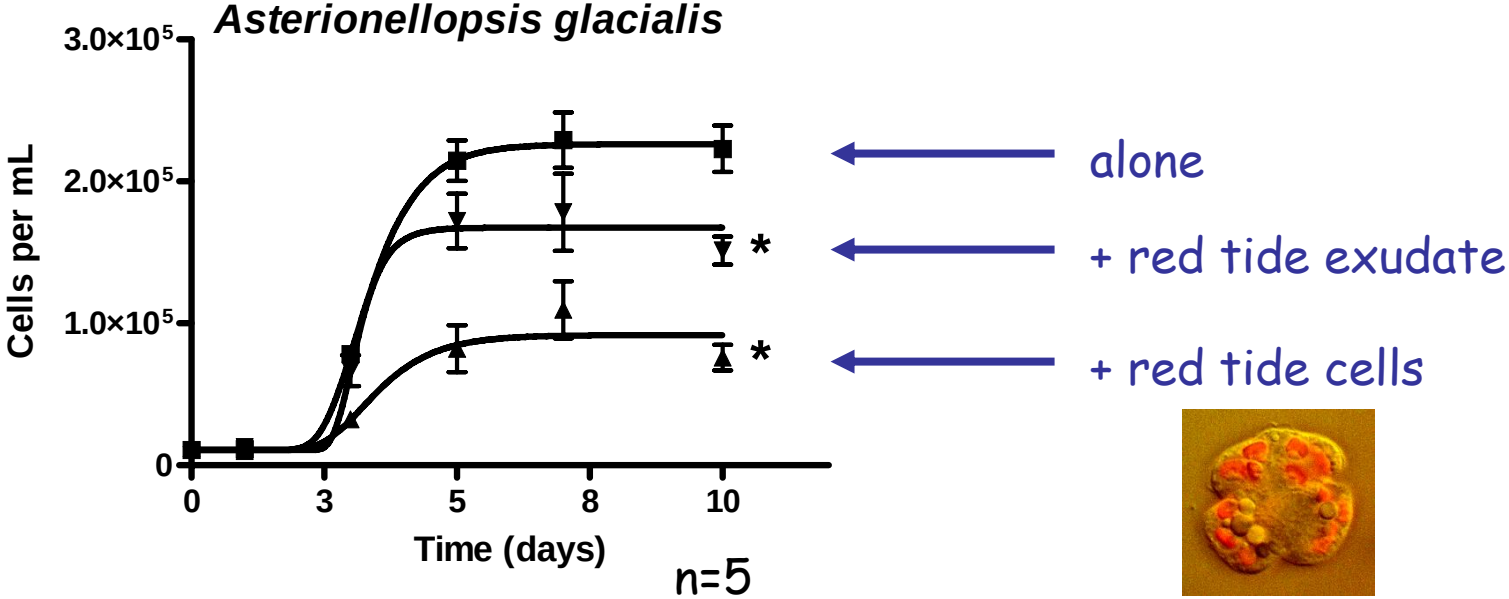
brevetoxin B (PbTx-2)

Kubanek, Hicks, Naar, Villareal (2005) Limnol. Oceanog. 50:883-895

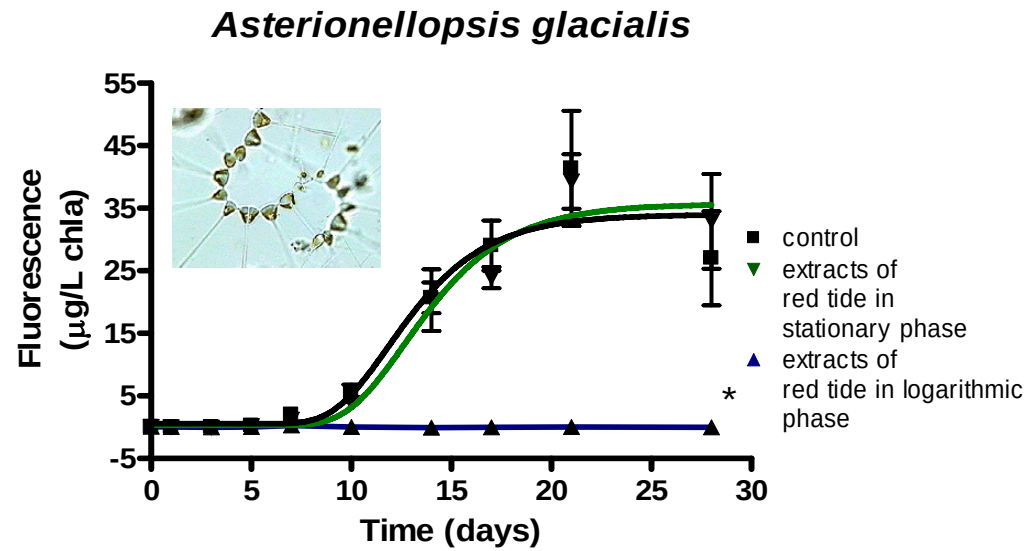
Growth of competing phytoplankton is suppressed by red tide cells and by red tide exudates

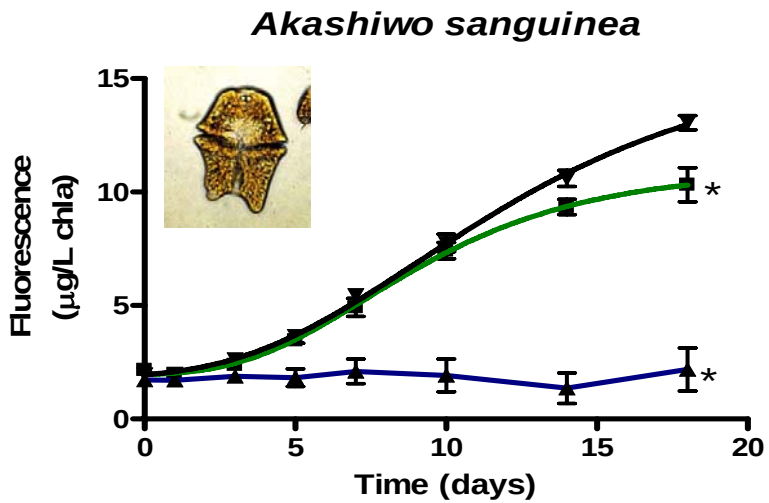


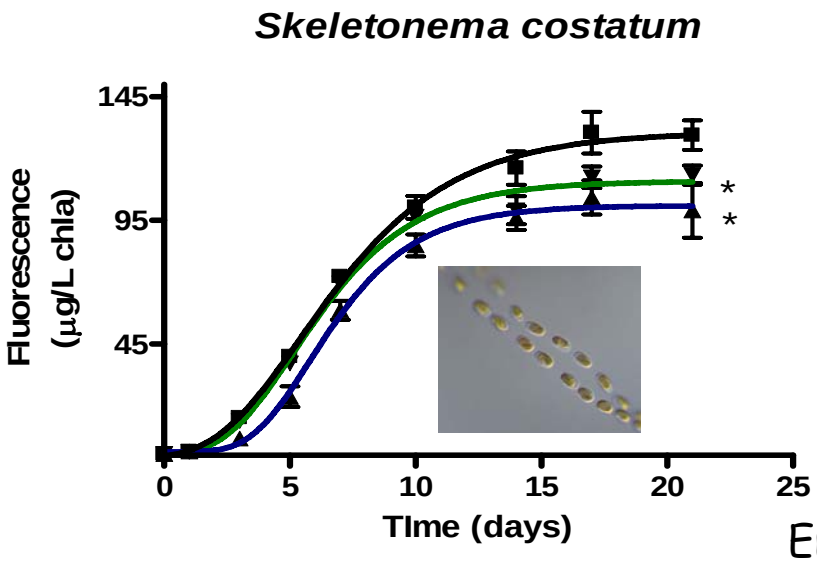
Asterionellopsis glacialis

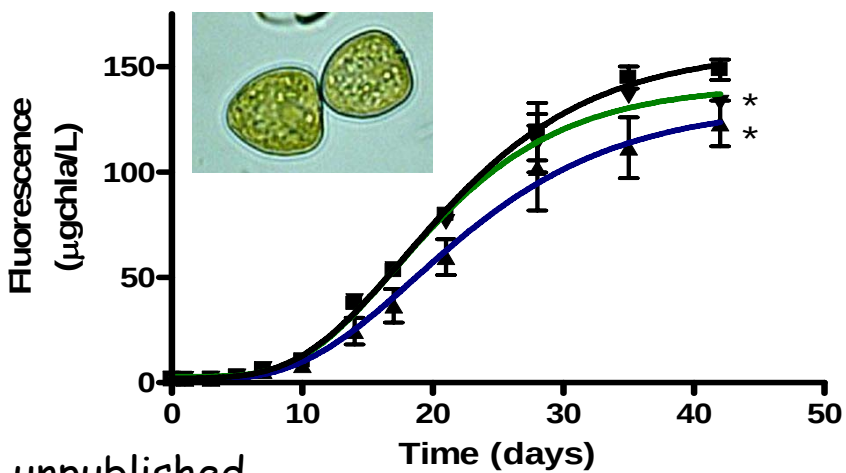


Strong evidence for red tide chemical warfare against several competitors



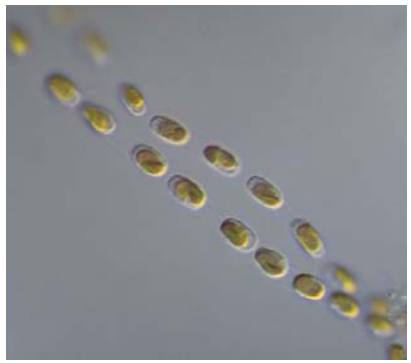


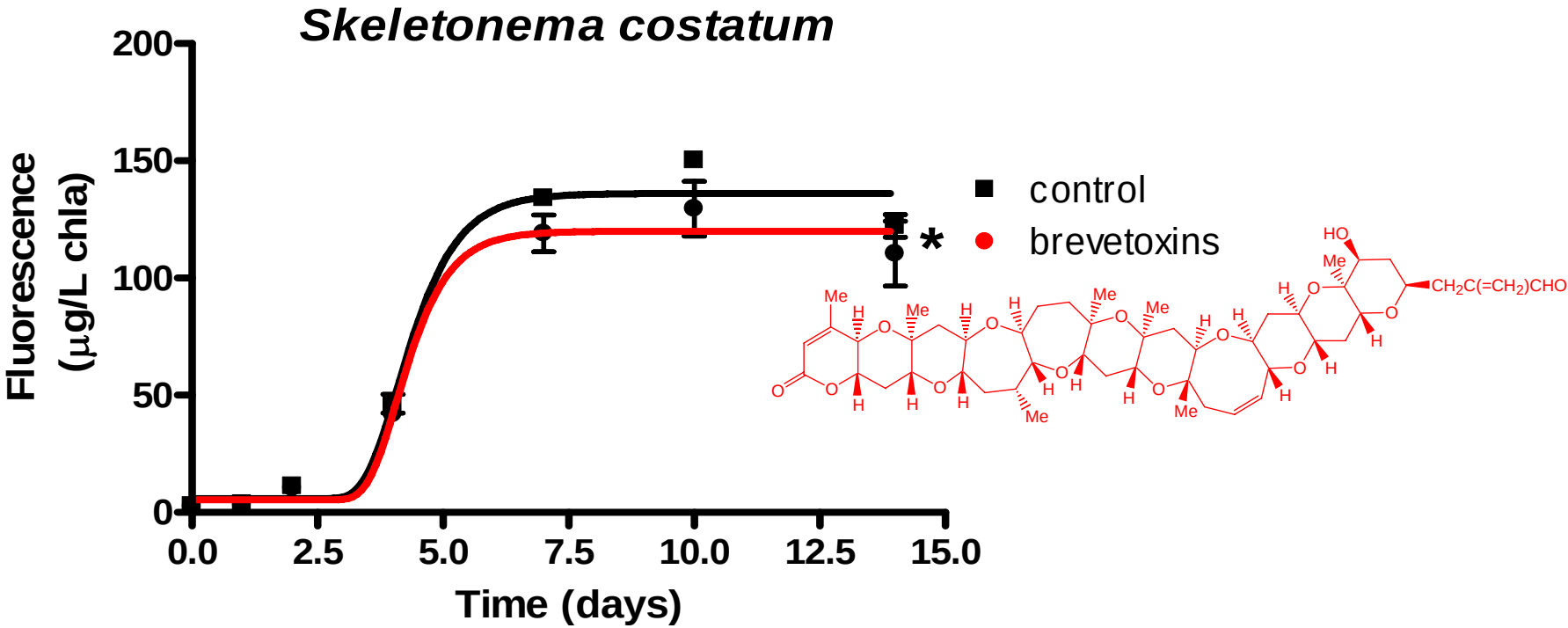




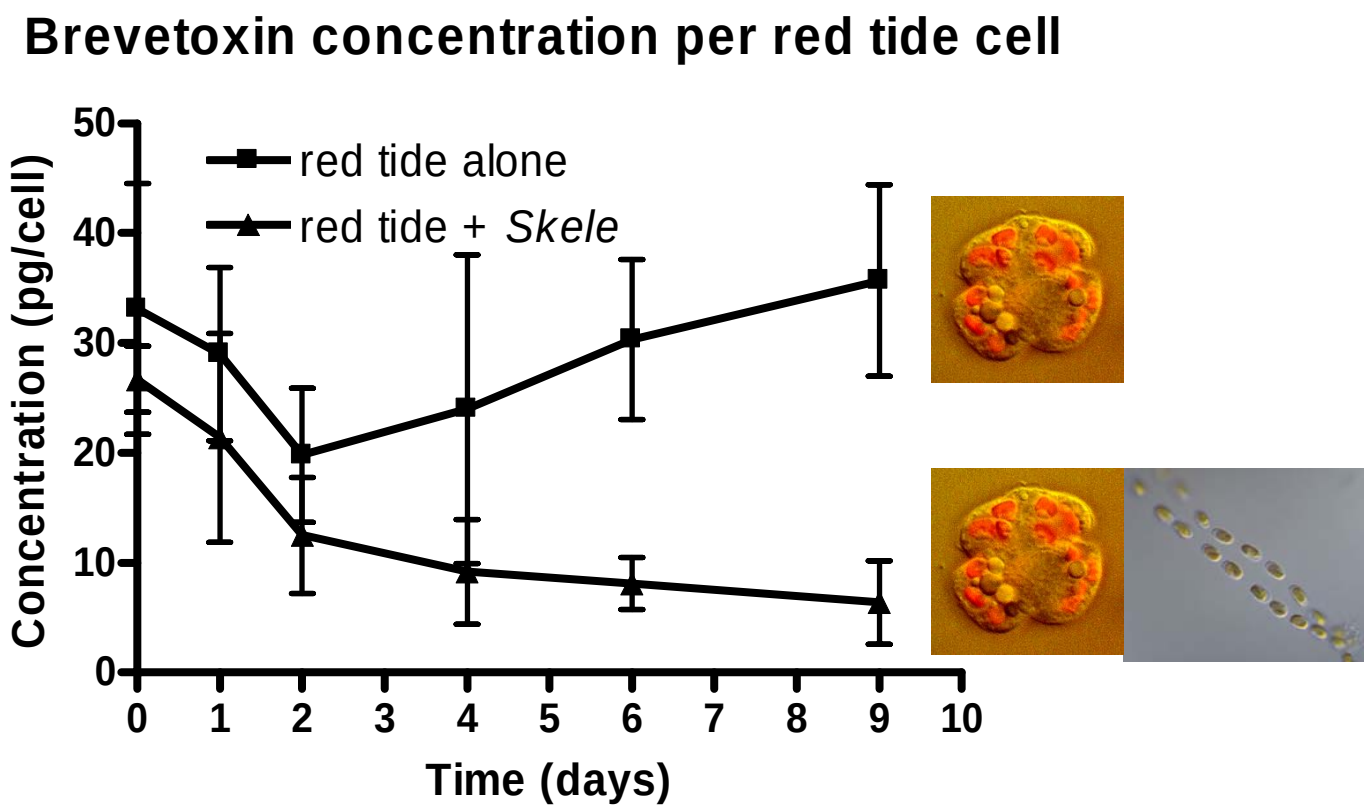
Emily Prince, unpublished

Red tide brevetoxins suppress competitor *Skeletonema costatum*



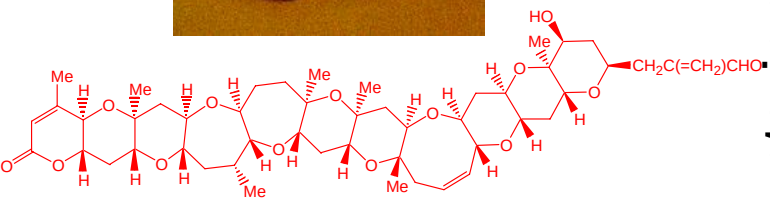


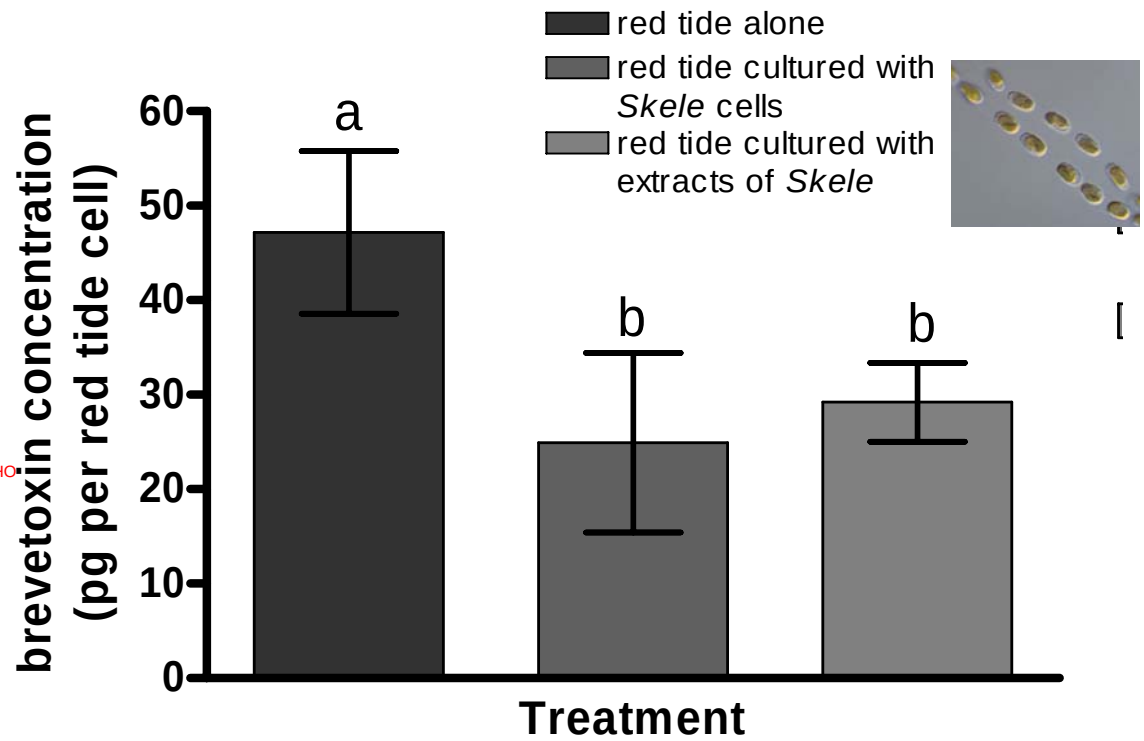
Skele fights back against red tide by inhibiting brevetoxin production



Tracey Myers & Emily Prince, unpublished

Skele produces a chemical inhibitor of red tide brevetoxins





We are working to identify this inhibitor…

Emily Prince & Tracey Myers, unpublished

Summarizing plankton chemical warfare:

- Florida red tide poisons its neighbors
- several (unknown) red tide compounds act against several competitors
- red tide brevetoxins suppress one important competitor (*Skele*)
- *Skele* fights back by inhibiting brevetoxin production by red tide cells
- interactions among planktonic organisms are complex!

Overall conclusions

- chemical cues are crucial in competitive, predator-prey, and host-pathogen interactions

- the molecules involved are structurally complex, regulated by subtle communication cues, and have specific targets

- the long-term persistence of populations and species, and therefore ecosystem function, is driven by these interactions

- we may be able to co-opt marine organisms' defenses and offenses in the search for new and better drugs

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